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Hydrogels Containing Monocaprin Prevent Intravaginal and Intracutaneous Infections With HSV-2 in Mice: Impact on the Search for Vaginal Microbicides

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Hydrogel formulations containing the 1-monoglyceride of capric acid (monocaprin) possess potent in vitro microbicidal activity against HIV and HSV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. These formulations were studied to determine whether they prevent intracutaneous and intravaginal infections of mice with HSV-2, a virus that is in vitro as sensitive to the virucidal action of the compound as is HIV. In mice intravaginal infection with HSV-2 and the associated mortality was prevented completely when the infection was carried out in the presence of a 20 mM monocaprin containing gel formulation. Similarly, virtually complete protection of lesion development and associated mortality was observed when mice were infected intracutaneously with HSV-2 in the presence of gels containing 10 or 20 mM monocaprin. No irritation or toxicity was observed following application of the gel to the skin or the vaginal mucosa. Hydrogel formulations of monocaprin could thus be pursued as vaginal microbicides for the prevention of sexual transmission of HSV, HIV and other infectious pathogens. ***J. Med. Virol. 61:107–110, 2000.***

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INTRODUCTION

Epidemiological and sociological research points to the importance of female controlled methods, e.g., vaginal virucides, to prevent or reduce the risk of sexually acquired infections, in particular infection with human immunodeficiency virus (HIV). The advantage of such preparations is that they may be used without the knowledge of the male sex partners. Vaginal microbicides would be very useful in countries where condom use, especially among sex workers, is not common. The virucide should: (i) cause very rapid inactivation of all strains of HIV and other sexually transmitted (ST) pathogens such as herpes simplex virus (HSV), Chlamydia and Neisseria; (ii) be non-irritant to the genital mucosa, without systemic toxicity and non-inhibitory to the normal vaginal flora; (iii) be active in vaginal fluids and semen; and (iv) be easy to apply. Gel formulations may in addition serve as lubricant and should be esthetically acceptable, stable in harsh climates and easily affordable.

Several safety and efficacy studies with the spermicide nonoxynol-9, a non-ionic surfactant with in vitro anti-HIV activity [Malkovsky, et al., 1988], have been reported. In a randomized, controlled trial in Nairobi, the use of nonoxynol-9 (1,000 mg) did not result in a reduced risk of HIV seroconversion compared with the use of a placebo cream [Kreiss et al., 1992]. Moreover, an increased risk of vulvar ulcers and vulvitis was observed suggesting that the frequent use of spermicides at high concentrations can increase the risk of HIV infection. A study carried out in Cameroon reported a lower rate of HIV infection among women who consistently used suppositories containing 100 mg nonoxynol-9 [Zekeng et al., 1993]. Sex workers in the same country reported that they like to use a nonoxynol-9-containing film, and over 80% of them stated they would continue to use it if it was shown to be effective

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against HIV and were to become widely available [Visness, et al., 1998]. In a study undertaken in Zambia no clear association between the use of nonoxynol-9 containing spermicides and the incidence of HIV infection was observed [Hira et al., 1997]. In a large double blind placebo controlled study, Roddy et al. [1998a,b] reported that use of an intravaginal film of 70 mg nonoxynol-9 did not protect against infection with HIV, Neisseria or Chlamydia. In addition, an increase in genital ulcers was observed in the nonoxynol-9-treated group, although this did not result in a higher incidence of HIV infection. By using a lower concentration (52.5 mg) of nonoxynol-9, Van Damme et al. [1998] observed only minimal toxicity after once daily use for 14 consecutive days. In contrast, the use of 100 mg nonoxynol-9, for 7 consecutive days, was found to be associated with irritation and evidence of inflammation, although the number of subjects was too small to allow statistical analysis [Stafford et al., 1998]. Finally, from a meta-analysis of other studies Cook and Rosenberg [1998] concluded that nonoxynol-9-containing spermicides may protect against infections with *Neisseria gonorrhea* and *Chlamydia trachomatis*, but that there are insufficient data to judge the effect on HIV transmission. Thus new microbicides that inactivate HIV and other ST pathogens and that have no adverse effects on the genital mucosa are urgently needed [Roddy et al., 1998b]. In this respect, it was reported recently on the virucidal activity of a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1, thiocarboxanilide UC-781 [Balzarini et al., 1998]. Several free fatty acids and 1-monoglycerides have been found to efficiently inactivate enveloped viruses [Thormar et al., 1987]. Hydrogel formulations that contain the 1-monoglyceride of capric acid (monocaprin) as the active ingredient rapidly destroy the infectivity of HIV-1, HSV-1, HSV-2, *Chlamydia trachomatis* and *Neisseria gonorrhoea* in vitro and are not toxic to the genital mucosa of rabbits by the standard rabbit vaginal irritation test [Thormar et al., 1999; Bergsson et al., 1998, 1999]. It was therefore of interest to study the efficacy of these gel formulations in preventing infection by viruses or bacteria in an animal model. We reported previously that the novel polysulfate PAVAS efficiently prevents experimental intravaginal and intracutaneous infection with HSV-2 in a murine model [Neyts et al., 1995]. Clinical studies with a gel containing a related molecule, dextrin sulphate [Stafford et al., 1997], are currently in progress. We report here that hydrogels containing monocaprin efficiently prevent intravaginal and intracutaneous HSV-2 infections in mice. In vitro HSV and HIV are about equally sensitive to the virucidal action of monocaprin. These findings have obvious implications in the search for vaginal microbicides for use in humans.

MATERIALS AND METHODS

Hydrogel Formulations

Hydrogel formulation 1A is based on a solution of sodium carboxymethylcellulose (NaCMC) and polyvinylpyrrolidone (Povidone K30) in water to which the 1-monoglyceride of capric acid (monocaprin) is added dissolved in glycofurol 75 (pH 7.0). Formulation 2A is based on a solution of hydroxypropylmethylcellulose (HPMC) and a carbomer (Carbopol 934) in water to which monocaprin is added. The pH is adjusted to 5.0 by addition of sodium hydroxide [Kristmundsdóttir et al., 1999]. The concentration of monocaprin in all formulations is 20 mM, except for "2A 10 mM MC" where it is 10 mM. NaCMC, glycofurol 75, monocaprin and Povidone K30 were purchased from Sigma Chemical Co. (St. Louis, MO). Carbopol 934 was from Nomeco (Copenhagen, Denmark), and HPMC from Aldrich Chemical Company Inc. (Milwaukee, WI). All of the ingredients were reagent grade.

TABLE I. Effect of Monocaprin in Gel Formulation 1A and 2A on HSV-2 Induced Vaginitis and Associated Mortality in Mice Infected Intravaginally With HSV-2

Condition	MDLI[a]	Number with lesion	MDD[b]	Survivors
1A control	3.7 ± 0.8	10/10	5.8 ± 0.6	0/10
1A 20 mM MC	—	0/10†	—	10/10†
2A control	3.4 ± 0.5	9/10	4.5 ± 0.7	1/10
2A 20 mM MC	—	0/10*	—	10/10*

[a]MDLI: mean day of lesion initiation.
[b]MDD: mean day of death.
*$P < 0.01$.
†$P < 0.001$.

Intracutaneous and Intravaginal Infection

The animals used throughout the experiments were adult (weighing ~20 g) female NMRI (Naval Medical Research Institute) mice and hairless (hr/hr) mice. The latter were bred at the Rega Institute by backcrossing and intercrossing of the homozygous parents. Both NMRI and hairless mice were housed under conventional conditions during the experiments. HSV-2 strain 196 was used in all experiments. Fifty microliter of the gel formulations was applied to the skin of anesthestized hairless mice. Scratches were then made with a scarificator in the skin of the lumbosacral area over a surface of about 1 cm^2. Mice were then inoculated intracutaneously in the scratched area with HSV-2 at $10^{3.7}$ plaque-forming units (PFU)/0.05 ml per mouse. Intravaginal infection of anesthestized NMRI mice was carried out by instillation of 50 μl of a virus suspension (10^4 PFU/ml) in the presence or absence of a hydrogel. An appropriate volume of the gel and virus (50:50 ratio) was mixed immediately before intravaginal inoculation. Mice were monitored daily. The significance of the numbers of survivors was calculated by the χ^2 test with Yates' correction. Statistical significance of the mean day of death (MDD) and mean day of lesion initiation (MDLI) was assessed by means of Student's *t*-test with Bonferroni correction.

RESULTS

It was first studied whether the gels were effective in preventing intravaginal HSV-2 infections (Table I). Because of the smallness of the mouse vagina, a mixture

TABLE II. Effect of Monocaprin on Intracutaneous HSV-2 Lesions and Associated Mortality in Hairless Mice When Added Before or After Infection

Condition	MDLI[a]	Number with lesion	MDD[b]	Survivors
No gel	4.0 ± 0.0	9/9	7.6 ± 1.3	0/9
Gel present during infection[c]				
2A control	5.0 ± 0.9[f]	7/9[e]	7.8 ± 1.5[e]	2/9[e]
2A 20 mM MC	8	1/9[h(g)]	11	8/9[h(f)]
2A 10 mM MC	—	0/4[f(f)]	—	4/4[f(e)]
Post infection treatment[d]				
2A control	5.6 ± 0.9[f]	5/5[e]	8.2 ± 0.7[e]	0/5[e]
2A 20 mM MC	5.8 ± 1.3[f(e)]	5/5[e(e)]	10.2 ± 2.8[e(e)]	0/5[e(e)]

[a]MDLI: mean day of lesion initiation.
[b]MDD: mean day of death.
[c]The gel was only present during infection.
[d]Treatment was initiated 6 hr post infection and was continued twice a day for the next 4 days.
[e]NS.
[f]$P < 0.05$.
[g]$P < 0.01$.
[h]$P < 0.001$.
[e,f,g,h]Letters without parentheses indicate the statistical significance between a certain group and the "no gel" condition. Letters between parentheses indicate the statistical significance between the monocaprin gel group and the corresponding control gel group.

of virus and gel had to be applied. If the gel was instilled before the viral inoculum the watery virus solution leaked out and no consistent infection was obtained in control animals receiving the inactive gels. Mixing of virus and gel was therefore done for each mouse individually seconds before inoculation. Mice that were inoculated intravaginally with virus mixed with control gel (i.e. without monocaprin), developed a vaginal infection, leading to paralysis of the hind legs and finally death. In contrast, none of the mice that received the virus mixed with a gel containing monocaprin developed any signs of infection. Intravaginal application of gels did not result in any signs of irritation or toxicity.

Because of the demonstrated efficacy of the gels in preventing intravaginal infections with HSV-2, the next study was to determine whether they would prevent intracutaneous HSV-2 infection. The hydrogel formulations were applied to the skin of hairless mice after which cutaneous lesions were made. The HSV-2 inoculum was then applied on the lesions followed by an additional gentle scarification. As shown in Table II, animals that had been pretreated with the control gels developed lesions as fast as the untreated control animals and died within the same time span. In contrast, all animals (except for one) that had been pretreated with gel 2A containing either 20 or 10 mM monocaprin were completely protected against the infection, i.e., no lesions developed nor was there, at any time, any sign of infection (Fig. 1). A toxicity study was undertaken in which gel 2A 20 mM was applied twice daily for 4 consecutive days to the skin of hairless mice. No signs of toxicity or irritation were observed macroscopically or histologically (data not shown).

DISCUSSION

Vaccines for preventing HIV transmission through mucosal immunity are unlikely to become soon available; and condoms, although highly effective in preventing HIV transmission, have failed to become generally accepted by males (e.g., clients of prostitutes) in many parts of the world. Vaginal microbicides have the advantage that their use can be controlled by the woman, if necessary without knowledge or consent of the male partner. Clinical trials with nonoxynol-9, a compound with in vitro virucidal activity against HIV [Malkovsky et al., 1988], show that the compound has a poor anti-HIV virucidal selectivity when applied intravaginally and may, depending on the dose used and the frequency of application, result in an increased risk of genital ulcers. Therefore, there is an urgent need for potent and selective virucidal agents that may function as alternative or secondary barriers for virus transmissions.

It was reported recently that hydrogel formulations containing 20 mM monocaprin are highly effective in in vitro inactivation of HIV-1, HSV-1, HSV-2, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. In the standard rabbit vaginal irritation test, the gels did not result in any adverse effects. In addition, the formulations appeared spermicidal [Thormar et al., 1998]. Because of (i) the lack of an animal model that allows to study protective measures against vaginal HIV transmission and (ii) the fact that HSV is, like HIV, an enveloped virus that is about as sensitive to the virucidal action of monocaprin as is HIV, we decided to study the effect of monocaprin-containing hydrogels on intravaginal infection with HSV-2 in mice. In addition, hairless mice were studied to determine whether the gel formulations are protective against an aggressive intracutaneous infection with HSV-2. In both model systems monocaprin-containing gels markedly protected mice against the infection. No signs of irritation or toxicity were observed either macroscopically or histologically. Healing of the scarified skin on which the monocaprin-containing gels had been applied was as fast as in the untreated controls.

Because of their (i) broad-spectrum and potent inactivating activity against sexually transmitted microorganisms, including HIV, HSV, *Neisseria* and *Chla-*

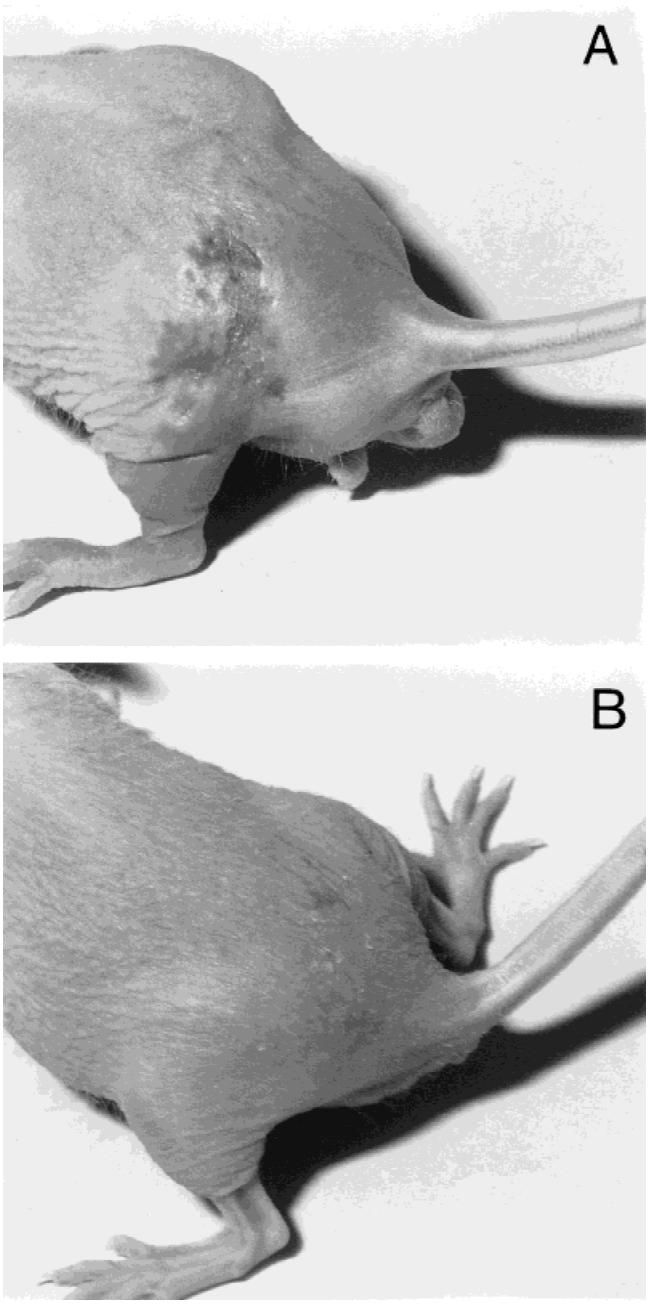

Fig. 1. Effect of pretreatment with gel 2A control (**A**) and gel 2A 20 mM MC (**B**) on intracutaneous HSV-2 infection in hairless mice as observed at 6 days post-infection.

mydia, (ii) potent activity in preventing intravaginal and intracutaneous infections with HSV-2 in mice, (iii) lack of irritation or toxicity for the vaginal mucosa or the skin of animals, and (iv) relative ease of application, gel formulations containing monocaprin should be considered further for development as vaginal microbicides.

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REFERENCES

Balzarini J, Naesens L, Verbeken E, et al. 1998. Preclinical studies on thiocarboxanilide UC-781 as a virucidal agent. AIDS 12:1129–1138.

Bergsson G, Arnfinnsson J, Karlsson SM, Steingríson O, Thormar O. 1998. In vitro inactivation of *Chlamydia trachomatis* by fatty acids and monoglycerides. Antimicrob Agents Chemother 42:2290–2294.

Bergsson G, Steingríson O, Thormar H. 1999. In vitro susceptibility of *Neisseria gonorrhoeae* to fatty acids and monoglycerides. Antimicrob Agents Chemother 43:2790–2792.

Cook RL Rosenberg MJ. 1998. Do spermicides containing nonoxynol-9 prevent sexually transmitted infections? A meta-analysis. Sex Transm Dis 25:144–150.

Hira SK, Feldblum PJ, Kamanga J, Mukelabai G, Weir SS, Thomas JC. 1997. Condom and nonoxynol-9 use and the incidence of HIV infection in serodiscordant couples in Zambia. Int J STD AIDS 8:243–250.

Kreiss J, Ngugi E, Holmes E, et al. 1992. Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. JAMA 268:477–482.

Kristmundsdóttir T, Arnadóttir SG, Bergsson G, Thormar H. 1999. Development and evaluation of microbicidal hydrogels containing monoglyceride as the active ingredient. J Pharm Sci 88:1011–1015.

Malkovsky M, Newell A, Dalgleish A. 1988. Inactivation of HIV by nonoxynol-9. Lancet i:645.

Neyts J, De Clercq E. 1995. Effect of polyanionic compounds on intracutaneous and intravaginal herpesvirus infection in mice: impact on the search for vaginal microbicides with anti-HIV activity. J Acquire Immune Defic Syndr Hum Retrovirol 10:8–12.

Roddy RE, Schulz KF, Cates W Jr. 1998b. Microbicides, meta-analysis, and the N-9 question: where's the research? Sex Transm Dis 25:151–153.

Roddy RE, Zekeng L, Ryan KA, Tamoufé U, Weir SS, Wong EL. 1998a. A controlled trial of nonoxynol 9 film to reduce male-to-female transmission of sexually transmitted diseases. N Engl J Med 339:504–510.

Stafford M, Cain D, Rosenstein I, et al. 1997. A placebo-controlled, double-blind prospective study in healthy female volunteers of dextrin sulphate gel. J Acquir Immun Defic Syndr Hum Retrovirol 14:213–218.

Stafford MK, Ward H, Flanagan A, et al. 1998. Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. J Acquir Immun Defic Syndr Hum Retrovirol 17:327–331.

Thormar H, Bergsson G, Gunnarsson E, et al. 1999. Hydrogels containing monocaprin have potent microbicidal activities against sexually transmitted viruses and bacteria in vitro. Sex Transm Infect 75:181–185.

Thormar H, Isaacs CE, Brown HR, Barshatzky MR, Pessolano T. 1987. Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides. Antimicrob Agents Chemother 31:27–31.

Van Damme L, Niruthisard S, Atisook R, et al. 1998. Safety evaluation of nonoxynol-9 in women at low risk of HIV infection. AIDS 12:433–437.

Visness CM, Ulin P, Pfannenschmidt S, Zeking L. 1998. Views of Cameroonian sex workers on a woman-controlled method of contraception and disease protection. Int J STD AIDS 9:695–699.

Zekeng L, Feldblum P, Oliver RM, Kaptue L. 1993. Barrier contraceptive use and HIV infection among high-risk women in Cameroon. AIDS 7:725–731.